H A N D O U T

Pathogens in Raw Milk: Prevalence and Clinical Manifestations and Raw Milk Associated Outbreaks and Food Borne Outbreak Investigations

Presentation to the Raw Milk Policy Working Group by Dr. Jim Kazmierczak

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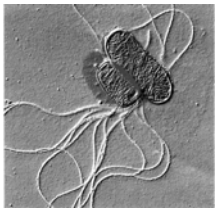
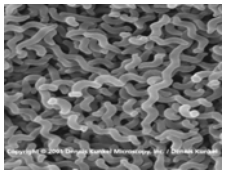
I. Selected Pathogens

Salmonella



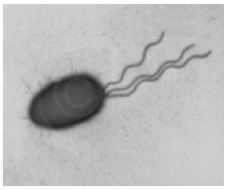
- Typical symptoms: Nausea, vomiting, diarrhea, abdominal cramps, fever, headaches, chills. Sepsis in immune compromised persons.
- Incubation period: 8-72 hours (18-36)
- Duration of illness: several days to several weeks, sometimes longer, asymptomatic shedding may last for weeks to months
- Characteristic foods: poultry, eggs, raw meats, unpasteurized milk
- Approx 900 cases annually in WI
- Prevalence in bulk milk: 8.9%[1], ~5% [2], 6%[3]

Campylobacter



- Typical symptoms: Nausea, bloody diarrhea, abd cramps, fever. ~1 per 1,000 can have a paralytic illness (Guillain-Barré Syndrome).
- Incubation period: 1-10 days (2-5)
- Duration of illness: up to a week or longer
- Characteristic foods: unpasteurized milk, poultry, water
- Other sources: puppies, kittens, cattle
- Low infectious dose (~ 500 organisms)
- Approx 1200 cases annually in WI
- Prevalence in bulk milk: 2%[3] , 9%[4], 12.3%[1]

E.coli O157:H7



- Typical symptoms: Diarrhea, bloody diarrhea, abdominal cramps, fever, headaches, chills, fatigue
- Incubation period: 12-60 hours (18-36)
- Duration of illness: several days to several weeks
- Characteristic foods: uncooked ground beef, unpasteurized milk
- Other sources: recreational water, cattle
- Low infectious dose (< 100 organisms)
- Approx 150 cases annually in WI
- Infection can result in hemolytic uremic syndrome (HUS) – damages blood vessels in the kidneys. 15% of infected children get HUS; 50% require dialysis; 5% die.
- Prevalence in bulk milk 3.8% [4], 2.4% [3]

Listeria

- Typical symptoms: Fever and diarrhea in immune competent; meningitis, blood stream infections in children and immune-compromised. Infection during pregnancy causes stillbirths or death of infant (30-50% case-fatality rate in newborns).

- Incubation period: 3-70 days
- Characteristic foods: soft cheeses, unpasteurized milk, ready-to-eat meats (cold cuts)
- Can grow well at refrigerator temperatures.
- Infectious dose < 1,000 organisms
- Approx 15 cases annually in WI
- Prevalence in bulk milk 2.8%,[3] 4.1% ,[1] 5% (WI study)[5] . Many of the WI isolates were resistant to multiple antibiotics[5]

General information on pathogens

1. Pathogens can be found in grass-fed, clean, healthy cows.[2,13] Some early studies indicated reduced shedding of *E.coli* O157:H7 when concentrate-based rations are eliminated, but additional studies have found that is not always true. Cattle which had been experimentally infected with E.coli O157:H7 and then fed on hay actually shed the bacteria longer than did grain-fed cattle (42 vs. 4 days)[11]. Another study observed that cattle fed a forage diet were O157:H7 culture-positive longer and with higher numbers of bacteria in their feces compared with cattle fed a grain diet.[12] Yet another study showed that cows with access to surface water and cows who grazed on roughage from fields on which manure had been spread had a higher incidence of *Salmonella*.[2] Importantly, the studies that did show some reduction in pathogen shedding with various diets almost never demonstrated elimination of shedding.

Table 1. Examples of recent foodborne disease outbreaks and recalls linked to "grass only, pastured" cattle. Provided courtesy of Dr. Michele Jay-Russell, University of California, Davis.

Year	State	Pathogen	Vehicle	Illnesses	Comment
2006	Multi	*E. coli* O157:H7	Baby spinach	205	Outbreak strain found in grassfed cattle feces and organically grown baby spinach
2006	CA	*E. coli* O157:H7	Raw milk/ raw colostrum	6	Non-outbreak strain of *E. coli* O157:H7 found in grassfed, organic heifers
2007	WA	*C. jejuni*	Raw milk	5	Outbreak strain found in grassfed milk
2007	CA	*C. jejuni*	Raw milk/ raw colostrum	11	Outbreak strain found in grassfed, organic cow feces
2007	CA	*Listeria*	Raw cream	No illnesses reported	*Listeria* found in grassfed, organic cream during routine testing
2008	CA	*C. jejuni*	Raw cream	No illnesses reported	*C. jejuni* found in grassfed, organic cream during routine testing
2009	CO	*C. jejuni*	Raw milk	81	PCR positive test for C. jejuni in grassfed raw milk

2. Presence of pathogens not always correlated with bacterial plate count [6,7]
3. Presence of pathogens not always assoc with somatic cell count – esp for coliforms [7,8]

4. Intermittent nature of shedding [2,9] – has implications when testing bulk milk.

II. Raw milk-associated disease and outbreaks

A. **National**: From 1993 through 2006, CDC received reports of 74 outbreaks associated with raw milk or its products. These involved 1,600 illnesses, 202 hospitalizations, and 2 deaths.

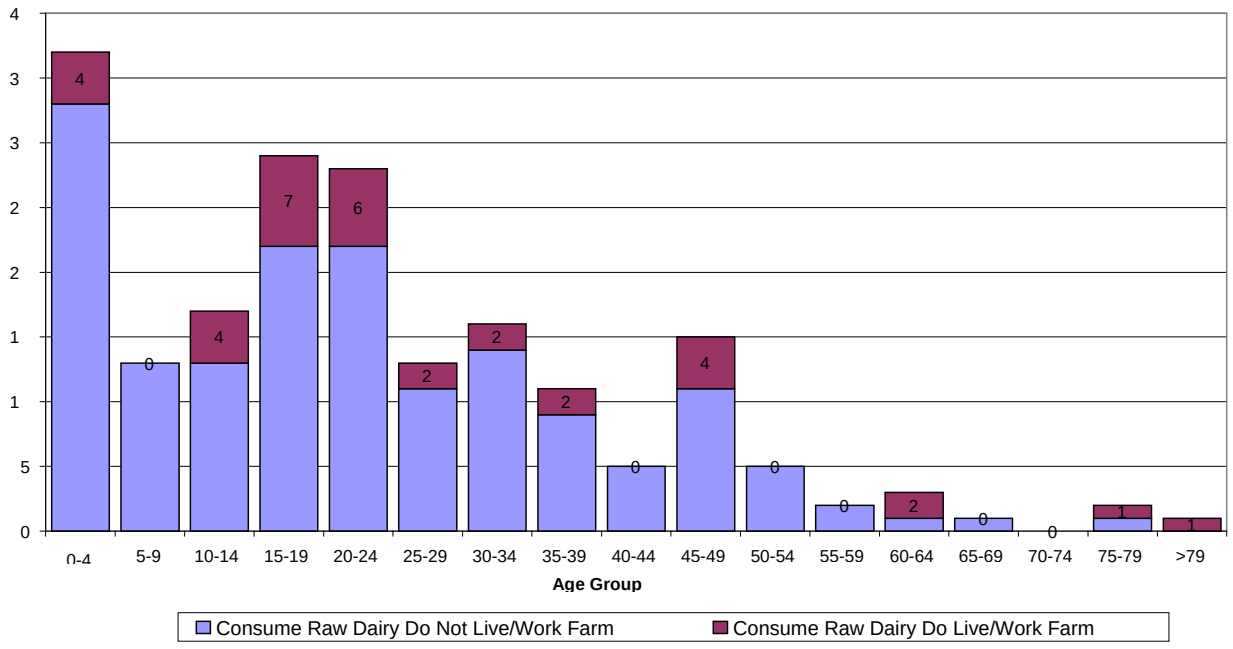
B. **Wisconsin**: Despite the prohibition on raw milk sales, the Wisconsin Division of Public Health has detected six raw milk-associated outbreaks since 1998: one *E. coli* O157:H7 and five *Campylobacter* outbreaks. Total of 261 cases; 27 hospitalized.

Year	County	Etiologic Agent	# Cases	# Hosp.	# Fatal	Preparation	Vehicle
1998	Chippewa	*E. coli* 0157:H7	63	24	0	Cheese factory	Unpast. cheese curds
2000	Walworth	*Campylobacter jejuni*	19	0	0	School - farm field trip	Unpast. milk
2001	Sawyer	*Campylobacter jejuni*	68	0	0	Dairy	Unpast. milk
2003	Rusk	*Campylobacter jejuni*	2	0	0	Farm	Unpast. milk
2006	Ashland	*Campylobacter jejuni*	58	2	0	Private home	Unpast. cheese curds
2009	Multi	*Campylobacter jejuni*	51	1	0	Farm	Unpast. milk

C. Sporadic (non-outbreak) illness – represents majority of cases

1. Reporting is mandated (labs, clinicians, hospitals), but surveillance is passive
2. Sporadic cases of illness resulting from raw milk consumption undoubtedly occur but are impossible to "tease out" of the background.
3. For example, during 2005-2007 in WI, 198 number of cases of campylobacteriosis reported having consumed raw dairy products within the 7 days prior to getting sick. (See figure below.) Cannot conclude that all 198 acquired infection from raw milk (Examples of other sources - contact with manure, kids in daycare, raw poultry). Because of this, percentage of cases acquired from raw milk is unknown.
4. Notable that 35 cases lived or worked on a farm. (Farmers are not invulnerable.)

2005-2007 Campylobacteriosis cases reporting unpasteurized dairy consumption, Wisconsin



D. Reasons for underreporting of cases of enteric disease and foodborne outbreaks

1. Patient(s) must be sick enough to see a doctor
2. Doctor must order testing
3. Positive results must get reported to public health
4. A common source may not be identified, esp if within a family, esp if small number of cases, esp if seen by different providers and cross public health jurisdictions.
5. Lack of cooperation with investigation
    a) Reluctance of case-patients to get a producer/neighbor "in trouble"
    b) Fear that reporting cases will lead to loss of supply source
    c) Refusal to believe that raw milk was causal
        i. Mail-based survey of 461 Ohio dairy farmers: 36% and 91% did not think that *Salmonella* and *Campylobacter* respectively, caused human disease.[10]
        ii. Survey of 248 PA producers: 32% were unaware that raw milk could contain disease-causing bacteria.[3]
6. Persons infected with these bacteria may not get overtly ill, but can still carry and shed these pathogens, and so may infect others, including persons who are immune-suppressed. These secondary cases would never be linked to a food source (e.g., situation in a daycare).

III. Overview of how DPH investigates a foodborne outbreak, PFGE

A. **Cases are reported** to health department (by lab, clinician, patients themselves)

B. Lab sends the patient's positive culture to State Lab of Hygiene for further analysis.

C. **Cases are interviewed** by LHD using standardized questionnaire - 7 day food hx obtained as well as questions about gatherings, hobbies, animal contact, etc.

D. Investigators **look for commonalities**. Common source outbreaks can be identified by:

1. history of a common food exposure, restaurant, social event, etc.
2. geographic or temporal clustering
3. "genetic fingerprinting" of the causative agent can be used to link cases (pulsed field gel electrophoresis or PFGE).

E. Implicating a particular food items:

1. Epidemiology:
    a. Sometimes, the evidence leaves no doubt about the vehicle – e.g., a food item that is typically consumed only infrequently is identified by a very high percentage of sick patients. (e.g., 37 of 40 ill ate alfalfa sprouts from one grower) If product still available, test food – usually at DATCP lab.
    b. May require a case-control study to implicate the vehicle of infection. Compares sick and well persons who all had a similar potential for exposure.

BAKED HAM:

Ill?	Yes (Ate)	No (Did not eat)	TOTAL
Yes	29	17	46
No	17	12	29
TOTAL	46	29	75

✓63% of those who ate ham got sick.
✓ 59% of those who did not eat ham got sick.
Statistical tests show no significant difference in risk between ham eaters and non-eaters.

ICE CREAM

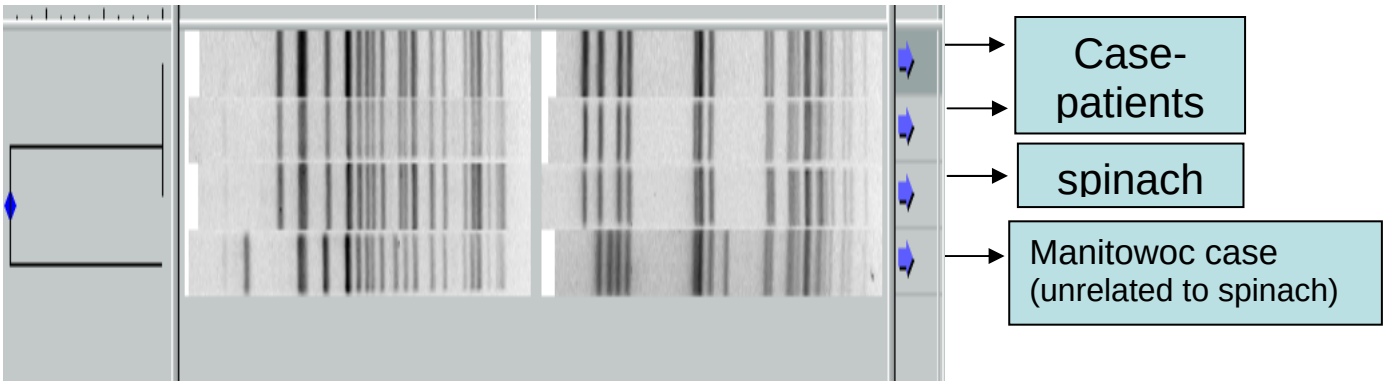
Ill?	Yes (Ate)	No (Did not eat)	TOTAL
Yes	43	3	46
No	11	18	29
TOTAL	54	21	75

✓ Almost 80% of those who ate ice cream got sick.
✓ Only 14% of those who did not eat it got sick.
Statistically, those who ate the ice cream had about 23 times the risk of getting sick versus those who did not have ice cream.

2. Testing food
    a. Done when a food item is implicated by epi investigation.
    b. Attempts to isolate a bacterial agent typically done by DATCP.
    c. If the same pathogen species is isolated from food as from the patients, culture transferred to SLH to compare with patient's isolate using PFGE.

3. **PFGE** = pulsed field gel electrophoresis (aka genetic fingerprinting)
    - Extract DNA from the isolated bacteria, chemically chop it into fragments
    - Put the chopped DNA into a gel, and apply electric current overnight.
    - DNA fragments migrate within the gel proportionate to their molecular weight.

Isolates with the outbreak strain (lanes 1-3) compared to an isolate from a different source (lane 4)



a. Very accurate way of linking bacterial isolates (i.e., which cases are actually part of an outbreak as well as those which are not related)

b. PFGE also important when relatively low # of cases that are not highly geographically clustered – can detect cases that are related.

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